α-TOCOPHEROL PROTECTS RAT BRAIN α-ADRENORECEPTORS AGAINST DAMAGE BY PHOSPHOLIPASE A_2

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The stabilizing action of vitamin E (α-tocopherol) in biological membranes is linked with its ability to interact with lipid radicals, to quench singlet molecular oxygen, to create order in the lipid bilayer of biomembranes, and to protect them against damage caused by the action of phospholipase A_2 [4, 6, 7, 8]. Protection against the damaging action of phospholipase A_2 in brain membranes is manifested, in particular, as the ability of α-tocopherol to stabilize the transmembrane potential and microviscosity of the synaptosomes during enzymic hydrolysis of their phospholipids by phospholipase A_2 [5]. Among the most important functional elements of brain membranes are their receptors, whose properties depend on the state of the lipid bilayer of the membranes, and can be changed, in particular, by the action of phospholipase A_2 [1, 2]. Accordingly, in the investigation described below the possibility of protecting brain membrane receptors against damage caused by phospholipase A_2 by α-tocopherol was studied, using β-adrenoreceptors as the example.

EXPERIMENTAL METHOD

The gray matter of the cerebral hemispheres of male Wistar rats weighing 120-140 g was homogenized in 0.32 M sucrose with the addition of EDTA (1 mM) and Tris (50 mM), pH 7.4. A glass—Teflon homogenizer (B. Braun, "West Germany") was used.

The membrane fraction of the brain cells was obtained by successive centrifugation (1000*g*, 17,000*g*) [3]. Synaptosomes, which are present in this fraction, were disintegrated by osmotic shock followed by freezing and thawing. A preparation of membrane vesicles, containing myelin and mitochondria as impurities, obtained by centrifugation at 17,000*g* was used in the experiments. The state of the β-adrenoreceptors was studied with the aid of the labeled antagonist ^{3}H-dihydroalprenolol ("Amersham," England). DL-propranolol ("Sigma," USA) was used as the competitive ligand. Vacuum filtration was carried out through GF/B filters ("Whatman," England). The quantity of bound labeled ligand was determined on a "RackBeta" liquid scintillation counter (LKB, Sweden), with counting efficiency of 30%. The state of the β-adrenoreceptors was judged by the specific binding level, and also after analysis of specific binding by Scatchard plot, relative to values of K_d and B_{max}. Membrane phospholipids were hydrolyzed with phospholipase A_2 (from bee venom, "Sigma," USA). The percentage of hydrolyzed phospholipids was measured by one-way thin-layer chromatography on high-efficiency LK5DF plates ("Whatman," England), followed by incineration in 10% H_2SO_4 solution in methanol, and quantitative automatic measurement of the peaks on a "Chromoscan-3" scanner (England). The α-tocopherol used in the work was obtained from "ICN Pharmaceuticals" (USA).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that treatment of the synaptosomal membranes with phospholipase A_2, when 2-5% of the membrane phospholipids were found to be hydrolyzed, lowered specific binding by 30%. Membranes treated with

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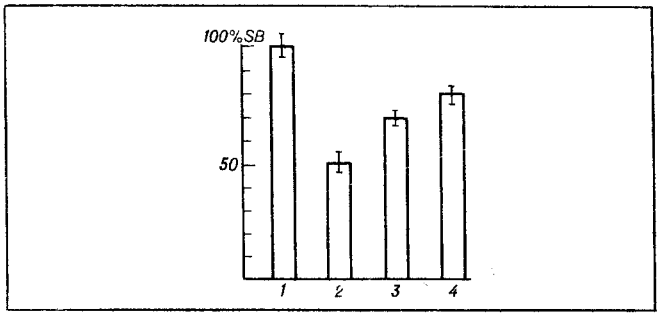

Fig. 1. Effect of α-tocopherol and phospholipase A_2 on binding of ^{3}H-dihydroalprenol; by synaptosomal membranes. Ordinate, binding of ^{3}H-dihydroalprenolol (in % of control). 1) Control; 2) membranes treated with α-tocopherol (20 nmoles/mg protein); 3) membranes treated with phospholipase A_2 (0.1 μg/mg protein, incubation for 1 min, 2-5% hydrolysis of membrane phospholipids); 4) membranes treated with α-tocopherol followed by phospholipase A_2.

TABLE 1. Effect of Phospholipase A_2 and α-Tocopherol on Parameters of Specific Binding of ^{3}H-dihydroalprenolol by Rat Brain Synaptosomal Membranes

Parameter	B_{max}, fmoles/mg protein	K_d, nM
Control	10,5±0,5	7,2±0,3
α-Tocopherol	5,2±0,5**	6,2±0,4*
Phospholipase A_2	6,4±0,3**	9,5±0,6*
α-Tocopherol + phospholipase A_2	8,3±0,3*	6,4±0,4

Legend. Experimental conditions correspond to those shown in caption to Fig. 1. Degree of significance of differences from control values: *$p < 0.01$; **$p < 0.001$.

α-tocopherol bound only half as much ^{3}H-dihydroalprenolol as in the control. Meanwhile, under the influence of phospholipase A_2, membranes preincubated with α-tocopherol increased their binding of ^{3}H-dihydroalprenolol (up to 80% of the control). Analysis of binding by Scatchard plot showed that the dissociation constant of ^{3}H-dihydroalprenolol in membranes treated with α-tocopherol was close to the control value (Table 1). Phospholipase A_2 increased dissociation of the ^{3}H-dihydroalprenolol — β-adrenoreceptor complex (K_d = 9.5 ± 0.6). If, however, the membrane vesicles were treated consecutively with α-tocopherol and phospholipase A_2, K_d came close to the control value; this may perhaps explain the increase in maximal binding of ^{3}H-dihydroalprenolol by β-adrenoreceptors under these conditions.

Thus α-tocopherol stabilizes the properties of brain membrane β-adrenoreceptors during hydrolysis of phospholipids by phospholipase A_2. This conclusion is in good agreement with the idea that the biological action of vitamin E is based on its ability to bind hydrolysis products of phospholipids by phospholipase A_2 as one of its molecular mechanisms [5].

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EFFECT OF BUSPIRONE AND BUSPIRONE-LIKE SEROTONIN 1A-AGONISTS ON SYSTEMIC BLOOD PRESSURE

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The role of serotoninergic neurons of the mesencephalic nuclei raphe in the regulation of systemic blood pressure (SBP), which was demonstrated in earlier work [2, 9], remains unclear, for serotonin, injected into the cerebral ventricles, lowers SBP in cats [2], but raises it in rats [9], whereas 8-hydroxy-2-(di-*n*-propylamino)tetraline, which selectively activates serotinin 1A receptors (R_{1A}-5-HT), lowers SBP exclusively both in cats [6, 10] and in rats [3, 4] when applied to the surface of the medulla [6] or injected intravenously [3, 4, 10]. Contradictory results have been obtained with other R_{1A}-5-HT activators, which are derivatives of 1-(2-pyrimidinyl)piperazine (1-FP), such as, for example, buspirone and ipsapirone [7, 10], which (buspirone) have been used or (ipsapirone, hepirone, campirone) studied experimentally and clinically as anxioselective agents.

This paper gives data on the effect of buspirone and some of its structural analogs and the active metabolite 1-PP [5] on SBP levels in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 110 noninbred albino rats weighing 200 ± 20 g, anesthetized with urethane (1 g/kg, intraperitoneally). SBP was measured in the common carotid artery by means of a mercury manometer Before and at various intervals of time after injection of the test drugs, the ECG was recorded in standard lead II on the ÉKP-03 M instrument. In some experiments the frequency and amplitude of the respiratory movements were recorded. Buspirone, ipsapirone (provided by the firm "Troponwerke," West Germany), campirone, levopirone and 1-PP (synthesized at the Institute of Physicoorganic Chemistry and Carbon Chemistry, Academy of Sciences of Ukrainian SSR), which were injected intravenously in doses of 0.1-30 mg/kg and in a volume of 0.1-0.3 ml. Pharmacological analysis of the changes in SBP and the chronotropic function of the heart was carried out with the aid of atropine sulfate, hexamethonium dibromide, cocaine hydrochloride, phentolamine hydrochloride, and metrazol (of USSR origin), picrotoxin (from "Merck," USA), deseryl ("Sandoz," Switzerland), and (±)-alprenolol ("Imperial Chemical Industries," Great Britain).

EXPERIMENTAL RESULTS

The majority of the tested substances modified SBP, depending on its initial level. In normotensive rats (70-120 mm Hg) all the substances tested caused a fall of SPB (Figs. 1 and 2). The effect developed immediately after injection and reached a maximum within 30-60 sec. Its degree and duration (3-40 min) depended on the dose of the drug. Effective doses,

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